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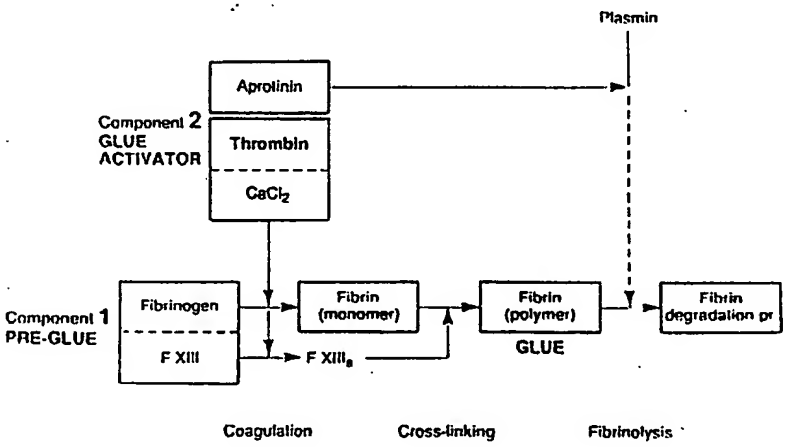

(57) Abstract

Method of preparing a cryoprecipitated suspension containing fibrinogen and Factor XIII useful as a precursor in the preparation of a fibrin glue which involves (a) freezing fresh frozen plasma from a single donor such as a human or other animal which has been screened for blood transmitted diseases at about -80°C for at least about six hours; (b) raising the temperature of the frozen plasma, e.g. to between about 0°C and room temperature, so as to form a supernatant and a cryoprecipitated suspension containing fibrinogen and Factor XIII; and (c) recovering the cryoprecipitated suspension. The invention also concerns a method of preparing a fibrin glue useful in surgical procedures which comprises: (a) preparing a cryoprecipitated suspension as described above; (b) applying a defined volume of the suspension to a desired site; and (c) applying a composition containing a sufficient amount of thrombin to the site so as to cause the fibrinogen in the suspension to be converted to the fibrin glue which then solidifies. The invention further concerns the cryoprecipitated suspension, the fibrin glue formed therefrom, fibrin glue kits and a method for sealing surgical wounds. Figure 1 illustrates the use of two components which exploits the final stage of coagulation. The first component contains fibrinogen and Factor XIII and is labeled "PREGLUE". The second component, labeled "GLUE ACTIVATOR", contains thrombin and may additionally contain apoprotinin and $CaCl_2$.

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FIBRIN ADHESIVE PREPARED AS A CONCENTRATE FROM SINGLE DONOR FRESH FROZEN PLASMA

Background of the Invention

This application is a continuation-in-part of U.S. Serial No. 648,752, filed September 7, 1984, the contents of which are hereby incorporated by reference into the present application.

Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

A major technical advance in surgery underutilized in this country has been the clinical application of fibrin glue. Clinical reports (1,3,8,9,10,12,14,16,17) document the utility of this concentrated adhesive, which duplicates the biological process of the final stage of normal coagulation (Figure 1). Fibrin glue has been used to control bleeding from liver lacerations and traumatized spleens (3,14). Other uses include sealing sewn or stapled tracheal and esophageal anastomoses as well as persistent air leaks or lacerations of the lung (1,12). Bronchial fistulas have been successfully closed using fibrin adhesive. A useful application has been in vascular surgery. An important feature is its ability to achieve hemostasis at vascular anastomoses particularly in areas which are difficult to approach with sutures or where suture placement presents excessive risk (1,10,12,16). Bleeding from needle holes or small arterial tears which cannot be controlled by suturing alone usually can be sealed by judicious fibrin glue ap-

plication (1). It has been especially helpful in obtaining hemostasis in heparinized patients or those with coagulopathy (1,17). Furthermore, fibrin glue impregnation permits the use of porous knitted grafts, even in anticoagulated patients, eliminating bleeding which has prevented the widespread use of these porous grafts in open heart surgery (1,8,9,16).

Various techniques have been described to pretreat porous vascular prostheses. Many are complicated, time consuming, expensive procedures and often render prostheses stiff and non-yielding (7,18). A previously described highly effective method using a cryoprecipitate preparation (6) was criticized because of its high cost (11).

Haverich et al. (8) reported that fibrin presealing allows the use of high porosity knitted Dacron prostheses even in heparinized patients. A highly porous fabric, with its superior healing characteristics, offers the potential for a lower incidence of right ventricular conduit obstruction (8). Fibrin presealed grafts are no more thrombogenic, and may be less so, than untreated grafts or those pretreated with blood (9,18). Highly porous fabrics have superior handling characteristics compared to low porosity grafts, and the use of fibrin adhesive could make low porosity woven Dacron grafts obsolete.

Despite generalized acceptance and use in Europe as a tissue sealant and hemostatic agent, fibrin glue has received little attention in the United States. In large part, this stems from the 1978 U.S. Food and Drug Administration ban (13) on the sale of commercially prepared fibrinogen concentrate made from pooled donors, e.g., as in Schwarz, et al., U.S. Patent Nos. 4,298,598 (1981), 4,362,567 (1982) or 4,414,976 (1983), because of the risk of transmission of

viral infection, in particular hepatitis B (2). In addition, the recent appearance of Acquired Immune Deficiency Syndrome also a major health concern, makes it unlikely that there will be a change in this policy in the foreseeable future (4).

Concentrated fibrinogen can be prepared with minimal risk of disease transmission from a patient's own blood (5,19). Although this technique obviously eliminates the risk of blood transmitted viral infection, it requires anticipating surgery at least two days in advance so that the autologous blood can be drawn and prepared in time. In addition, it requires the patient to donate at least one unit of blood and may result in the need for blood transfusion to replace donated blood. Furthermore, it is not practical to depend on autologous blood as a source for fibrin adhesive in trauma cases and other unanticipated surgical emergencies. This invention concerns a convenient and practical method of preparing fibrinogen and fibrin glue which avoids the risk of transmission of disease in contrast to prior methods, e.g., the method of Schwarz, et al. This method makes available an abundance of fibrinogen concentrate safe for use within minutes whenever needed in the operating room.

Summary of the Invention

This invention concerns a method of preparing a cryoprecipitated suspension containing fibrinogen and Factor XIII useful as a precursor in the preparation of a fibrin glue. The method involves (a) freezing fresh frozen plasma from a single donor, e.g. a human or other animal such as a cow, pig or sheep, which has been screened for blood transmitted diseases, e.g. one or more of syphilis, hepatitis or acquired immune deficiency syndrome at about -80°C for at least about 6 hours, preferably for at least about 12 hours; (b) raising the temperature of the frozen plasma, e.g. to about 0°C to about room temperature, preferably to about 4°C, so as to form a supernatant and a cryoprecipitated suspension containing fibrinogen and Factor XIII; and (c) recovering the cryoprecipitated suspension, e.g. by decanting the supernatant. The suspension may be concentrated, e.g., by centrifugation.

This invention further concerns the cryoprecipitated suspension containing fibrinogen and Factor XIII so prepared, which additionally may be pre-formed and stored frozen.

The invention also concerns a method of preparing a fibrin glue useful in surgical procedures which involves (a) preparing a cryoprecipitated suspension as described above; (b) applying a defined volume of the suspension to a desired site; and (c) applying a composition containing a sufficient amount of thrombin from an appropriate source, e.g. human, bovine, ovine or porcine thrombin, to the site so as to cause the fibrinogen in the suspension to be converted to the fibrin glue which then solidifies in the form of a gel.

The thrombin-containing composition may also contain a suitable amount of an anti-fibrinolytic substance, such as

apoprotinin and may also contain $CaCl_2$.

The suspension and the composition containing thrombin may be applied to the site in a surgically acceptable vehicle such as a gel, e.g. gelatin or a stretched fabric.

The invention further concerns a method of sealing a surgical wound which involves applying to the wound a suitable amount of a cryoprecipitated suspension prepared in accordance with this invention and applying a composition containing thrombin to the site so as to cause the fibrinogen in the suspension to be converted to a fibrin glue which then solidifies in the form of a gel. Pressure may be applied to the fibrin glue until it solidifies. The suspension and composition may be applied in a surgically acceptable vehicle such as a gel. Alternately, they may be applied in a stretched fabric such as a synthetic vascular patch or graft.

Additionally, this invention involves a fibrin glue kit for use in providing hemostasis during surgery. The kit contains the above-mentioned cryoprecipitated suspension and a composition containing thrombin. The thrombin-containing composition may additionally contain a suitable amount of an anti-fibrinolytic substance, e.g. apoprotinin, and may also contain a suitable amount of an appropriate calcium salt, e.g. $CaCl_2$. The kit may further contain at least one synthetic vascular graft or patch.

Brief Description of the Figures

Figure 1: The use of fibrin adhesive exploits the final stage of coagulation cascade. The first component which is prepared from fresh frozen plasma (FFP) by cryoprecipitation contains fibrinogen and Factor XIII (FXIII) and is labeled "PREGLUE". The second component, labeled "GLUE ACTIVATOR" contains thrombin and may additionally contain Apoprotinin and $CaCl_2$.

Detailed Description of the Invention

As mentioned above, this invention concerns a method of preparing a cryoprecipitated suspension containing fibrinogen and Factor XIII useful as a precursor in the preparation of a fibrin glue which comprises:

a) freezing fresh frozen plasma from a single donor which has been screened for blood transmitted diseases such as one or more of syphilis, hepatitis or acquired immune deficiency syndrome at about -80°C for at least about 6 hours, preferably for at least about 12 hours;

b) raising the temperature of the frozen plasma to between about 0°C and room temperature, preferably about 4°C, so as to form a supernatant and a cryoprecipitated suspension containing fibrinogen and Factor XIII; and

c) recovering the cryoprecipitated suspension.

In a preferred embodiment the cryoprecipitated suspension is prepared in 50 cc polypropylene centrifuge tubes (Fisher Scientific, Springfield, NJ) which have been charged with screened, fresh frozen plasma (FFP) from a single donor. The single donor may be a human or an animal, e.g. a cow, sheep, pig, goat, rabbit, guinea pig, rat or mouse so long as the cryoprecipitated suspension is capable of reacting with thrombin from an appropriate source to produce fibrin glue, as disclosed herein. Preferably the fresh frozen plasma is bovine, ovine or porcine. The tubes are then placed in a freezer at -80°C for at least twelve hours, and the fibrinogen-containing suspension prepared for use or storage by thawing over several hours at 4°C. The tubes containing the thawed fibrinogen-containing suspension are then centrifuged, e.g., at about 1000-2300 x G for fifteen to twenty

minutes preferably in a refrigerated centrifuge. The supernatant is decanted leaving a yellowish precipitate containing fibrinogen. The precipitated and centrifuged pellet of concentrated fibrinogen is resuspended in the small amount of supernatant remaining after decantation.

Since the final concentration of fibrinogen is partly determined by the volume of residual supernatant which is used to resuspend the fibrinogen pellet, this maneuver is performed with care so as to remove as much liquid as possible without disturbing the pellet. Next, the concentrated fibrinogen suspension is aspirated into a syringe using a large bore spinal needle. The total yield is approximately 2 cc's of concentrated fibrinogen from each 40 cc's of FFP. Measured fibrinogen in this concentrate is 2160 mg% compared to 260 mg% in the virgin FFP (see Table I). Factor XIII also is present in the final preparation. All aspects of the procedure are performed in a laminar flow hood under sterile conditions and the final product is checked for contamination by routine culture.

The concentrated fibrinogen-containing cryoprecipitated suspension may be prepared well in advance of its intended use, i.e., pre-formed, and stored for up to two months at -80°C before use. Reports indicate it can be stored for as long as one year (5). After thawing, it reportedly can be kept for three to four days at 4°C, but we have found it retains biological activity for as long as two weeks. Once at room temperature, the concentrate should be used within four hours (5).

This invention further involves a method of preparing a fibrin glue useful in surgical procedures which comprises: (a) preparing a cryoprecipitated suspension as described above; (b) applying a defined volume of the suspension to a

Table I. Fibrinogen, Factor XIII, and Fibrin Split Product Levels in Representative Samples of Fresh Frozen Plasma and Whole Blood

Sample	FSP (g/ml)	Factor XIII	Fibrinogen (mg%)
FFP-A[1]	--	present	260
FFP-A[2]	0	present	2160
Whole Blood[3]	0	present	2160

[1] A sample of FFP was thawed only and then tested without cryoprecipitation treatment.

[2] Cryoprecipitate of sample A revealing almost a 10 fold increase of concentration.

[3] Unit of single donor whole blood obtained from Presbyterian Hospital Blood Bank where it had been stored for several days at 1-4°C.

desired site; and (c) applying a composition containing a sufficient amount of thrombin to the site so as to cause the fibrinogen in the suspension to be converted to the fibrin glue which then solidifies in the form of a gel.

Suitable thrombin for use in this invention may be derived from a human or an animal, e.g. a cow, sheep or pig and includes e.g. human, porcine or bovine thrombin such as commercial bovine thrombin, e.g. Thrombinar® (Armour Pharmaceutical Co., Kankakee, IL). The thrombin-containing composition may also contain a suitable amount of an anti-fibrinolytic substance, e.g. apoprotinin, and a suitable amount of an appropriate calcium salt, e.g. $CaCl_2$.

In a preferred embodiment the glue is prepared as follows: the cryoprecipitated suspension containing fibrinogen and Factor XIII and commercial bovine thrombin (Thrombinar® Armour Pharmaceutical Co., Kanakee, IL) are placed into separate one cc syringes. A defined volume of the fibrinogen-containing cryoprecipitated suspension is applied to a desired site. The composition containing a sufficient amount of thrombin is then applied to the site so as to cause the fibrinogen in the suspension to be converted to the fibrin glue which then solidifies in the form of a gel. With a thrombin mixture of 500 units/ml, the fibrinogen gels into a fibrin clot in less than one minute. The glue works most effectively when applied in a relatively dry field. For example, application to a vascular suture line several minutes prior to clamp removal allows time for the glue to congeal. A transparent gel-like film adheres to the suture line and results in hemostasis after the clamps are released. In a wet field, the suspension and the composition containing thrombin may be applied to the site in a surgically acceptable vehicle. In a wet field a vehicle comprising a gel such as gelatin, e.g. Gelfoam® (Upjohn

Co.), or microfibrillar collagen wafers (collagen fleece) e.g. Avitine® (Alcon, Inc., Humacao, Puerto Rico) is preferred. Preferably the collagen is bovine, ovine or porcine collagen. In the former embodiment the gel is saturated with thrombin and then impregnated with the fibrinogen. The Gelfoam® acts as a vehicle to hold the fibrin while clotting occurs. To control active bleeding, the glue should be applied on thrombin soaked Gelfoam® and digital pressure exerted over the site of bleeding for one minute while the glue sets. Care must be taken to avoid suctioning directly over the area of glue application because this may result in aspirating not only blood, but also soluble glue before it has polymerized.

To pretreat porous vascular grafts, the technique described by Borst (1) may be employed using a vehicle comprising a stretched fabric. In this embodiment several milliliters of the fibrinogen concentrate is spread over the outer surface of the stretched fabric. Following thorough coating of the graft, the thrombin activating solution is massaged into the graft. After the graft is inserted, a few additional drops of glue are applied directly over needle holes before clamp removal. Porous grafts, so pretreated, offer advantages in handling, suturing and long-term patency.

As with any technique, successful use of fibrin glue depends on proper application gained through experience. Our adhesive has stopped bleeding around vascular anastomoses, particularly needle holes and small linear tears, in a variety of situations involving aortotomies, reversed saphenous vein graft aortic and coronary artery anastomoses, and right ventricular conduit suture lines. By helping to control difficult bleeding, the use of fibrin glue in accordance with this invention can decrease the need for blood transfusions, shorten operating room time and may even

be lifesaving. We have found that as experience is gained with the glue the list of potential uses is expanding.

Fibrin glue can be prepared from single donor FFP in sufficient quantity to meet surgical demand because the ease of extraction permits making large amounts of the glue. It can be stored for long periods of time at -80°C or for shorter periods of time at 4°C until it is needed. By the method of this invention, it is not necessary to anticipate an operation days in advance in order to have fibrin glue available. It has become our practice to maintain a supply of glue at 4°C for immediate use. This stock is checked and updated periodically.

One unit of FFP yields eight to ten cc's of concentrated fibrinogen. This represents enough glue to use in several operations. Approximately two to three cc's of concentrated cryoprecipitated suspension containing the fibrinogen combined with an equal volume of thrombin is adequate to preseal a right ventricular conduit. As little as one cc of glue effectively achieves hemostasis of bleeding suture lines or needle holes. In comparison, when fibrinogen is prepared from autologous whole blood, a smaller volume is extracted (approximately one cc for every one hundred cc's of whole blood) and it is limited to the volume of blood donated by that individual.

Moreover, the method of this invention of preparing the cryoprecipitated fibrinogen-containing suspension and the fibrin glue is easy to learn, reproducible, and economical. Most importantly, the use of single donor FFP entails no greater risk of transmission of Hepatitis B, Acquired Immune Deficiency Syndrome, and other serologically transmitted illness than transfusion of a unit of fresh frozen plasma. This decreased risk of blood born infection should cir-

cumvent the risk of blood transmitted diseases which led to the FDA proscription against preparing fibrin concentrate from pooled donors. Fibrin glue in accordance with this invention could become as readily available in this country as the preparation from pooled blood has been in Europe.

Comparison of fibrinogen prepared from autologous whole blood with the lyophilized, commercial European product (Tissucol®, Immune AG, Vienna, Austria) indicates the tear coefficient of the latter is stronger (5). Unlike Tissucol®, one embodiment of the present invention lacks antifibrinolytic additives and calcium chloride in the activating solution. The tenfold concentration of fibrinogen and presence of assayable Factor XIII indicate the hemostatic potency of our preparation. When used as a hemostatic agent or graft sealant, the FFP-derived glue functions so well that its adhesive properties are clear and unequivocal.

The cryoprecipitate of this invention can be included in a fibrin glue kit which also includes a composition containing thrombin. The thrombin-containing composition may additionally include a suitable amount of an appropriate calcium salt such as $CaCl_2$ or an anti-fibrinolytic substance such as apoprotinin or both. The kit may also include a surgically acceptable vehicle for the fibrin glue, such as a gel, or at least one synthetic vascular graft or patch or both. In particular, highly porous knitted grafts which appear to have long term patency rates superior to other types of grafts, but cannot presently be used in anti-coagulated patients (because of uncontrollable life threatening bleeding) could become widely utilized through this technique and may be included in a fibrin glue kit of this invention. It also may be possible to employ smaller synthetic fabric grafts in peripheral vascular surgery, currently not used because of

high occlusion rates.

The methods, materials or kits of this invention may be used for sealing a surgical wound by applying to the wound a suitable amount of a cryoprecipitated suspension of this invention and applying a composition containing thrombin to the site so as to cause the fibrinogen in the suspension to be converted to a fibrin glue which then solidifies in the form of a gel. Pressure may be applied to the fibrin glue until it solidifies. The suspension and composition may be applied in a surgically acceptable vehicle, e.g. a gel. They may also be applied in a stretched fabric such as a synthetic vascular graft or patch. Uses for the methods, materials or kits of this invention in vascular surgery include providing hemostasis for stitch hole bleeding of distal coronary artery anastomoses; left ventricular suture lines; aortotomy and cannulation sites; diffuse epimyocardial bleeding seen in reoperations; and oozing from venous bleeding sites, e.g. at atrial, caval, or right ventricular levels. The invention is also useful for stopping bleeding from damaged spleens (thereby saving the organ), livers, and other parynchymatous organs; sealing tracheal and bronchial anastomoses and air leaks or lacerations of the lung; sealing bronchial stumps, bronchial fistulas and esophageal fistulas; for sutureless seamless healing ("Zipper" technique), and embolization in vascular radiology of intracerebral AVM's, liver AVM's, angiodysplasia of colon, esophageal varices, "pumping" GI bleeders secondary to peptic ulcers, etc. This invention is further useful for providing hemostasis in corneal transplants, nosebleeds, post tonsillectomies, teeth extractions and other applications.

EXAMPLE 1

Preparation of Cryoprecipitated Suspension (Pre-Glue)

FFP or citrated whole blood is obtained at least about 18 hours before intended use of Pre-glue. If whole blood is used as the starting material, it is first centrifuged at 2300 x G for 5 min in 50 ml polypropylene centrifuge tubes (Fisher Scientific, Springfield, NJ). The plasma is then aspirated carefully, so as not to disturb the buffy coat or RBC layer. The plasma so prepared or FFP is then frozen at -80°C for at least 12 hours. The frozen plasma is then thawed slowly at 4°C (or at room temperature, though 4°C is preferable). After thawing the tubes are centrifuged again at 2300 x G for 20 minutes in a refrigerated centrifuge to separate the fluffy cryoprecipitate. The tubes are removed from the centrifuge carefully, so as not to resuspend the pellet. The supernatant is carefully decanted. Tapping the tubes resuspends the cryoprecipitate in the remaining plasma. The pellet, which contains fibrinogen and FXIII, is thick and yellow, and is aspirated into a syringe with a spinal needle for use or transfer to storage. All equipment used in the above-described example must be sterile.

EXAMPLE 2

Preparation of Fibrin Glue

A drop of the cryoprecipitated suspension prepared as in Example 1 is placed at the desired site. An amount of a composition containing reconstituted thrombin (e.g. Thrombinar®, Armour Pharmaceutical Co., Kankakee, IL) appropriate to the amount of fibrinogen used is then added to the desired site. The thrombin causes the fibrinogen to be converted into the fibrin glue which solidifies in the form of a gel in less than one minute.

EXAMPLE 3

Preparation of Bovine Cryoprecipitated Suspension (Bovine Pre-Glue)

Example 1 may be repeated using fresh frozen bovine plasma or citrated bovine whole blood instead of human FFP or citrated human whole blood. Bovine pre-glue may be thereby obtained.

EXAMPLE 4

Preparation of Bovine Fibrin Glue

Example 2 may be repeated using bovine pre-glue prepared as in Example 3 in place of cryoprecipitated suspension of Example 1.

EXAMPLE 5

Use of Fibrin Glue without
Anti-Fibrinolytic Additives

Fibrin glue prepared by the method of Example 2 was used to cover exposed saphenous vein grafts in a patient with a sternal wound infection which was treated with open drainage. The glue was monitored and was still in place 48 hours after application. The patient had received multiple wet to dry dressing changes and water pick irrigations of the wound without dislodgement of the glue. No evidence of gross fibrinolysis, e.g. late bleeding, was observed. Since the glue was formed without an antifibrinolytic additive such as apoprotinin, such additives do not appear to be essential.

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What is Claimed is:

1. A method of preparing a cryoprecipitated suspension containing fibrinogen and Factor XIII useful as a precursor in the preparation of a fibrin glue which comprises:

a) freezing fresh frozen plasma from a single donor which has been screened for blood transmitted diseases at about -80°C for at least about 6 hours;

b) raising the temperature of the frozen plasma so as to form a supernatant and a cryoprecipitated suspension containing fibrinogen and Factor XIII; and

c) recovering the cryoprecipitated suspension.

2. A method of claim 1, wherein the donor is a human or other animal.

3. A method of claim 2, wherein the donor is a cow, sheep or a pig.

4. A method of claim 1, wherein the blood transmitted diseases comprise one or more of syphilis, hepatitis or acquired immune deficiency syndrome.

5. A method of claim 1, wherein the fresh frozen plasma is frozen in step (a) for at least about 12 hours.

6. A method of claim 1, wherein the temperature of the frozen plasma is raised in step (b) to between about 0°C and about room temperature.

7. A method of claim 1, wherein recovering the cryoprecipitated suspension comprises decanting the supernatant

from the cryoprecipitated suspension.

8. A method of claim 1 which further comprises concentrating the cryoprecipitated suspension by centrifugation.

9. A cryoprecipitated suspension containing fibrinogen and Factor XIII prepared by the method of claim 1.

10. A pre-formed frozen cryoprecipitated suspension of claim 9.

11. A method of preparing a fibrin glue useful in surgical procedures which comprises:

a) preparing a cryoprecipitated suspension according to claim 1;

b) applying a defined volume of the suspension to a desired site; and

c) applying a composition containing a sufficient amount of thrombin to the site so as to cause the fibrinogen in the suspension to be converted to the fibrin glue which then solidifies in the form of a gel.

12. A method of claim 11, wherein the thrombin is derived from a human or other animal.

13. A method of claim 12, wherein the animal is a cow, sheep or pig.

14. A method of claim 11, wherein the composition also contains a suitable amount of an anti-fibrinolytic substance.

15. A method of claim 14, wherein the anti-fibrinolytic substance is apoprotinin.

16. A method of claim 11, wherein the composition also contains a suitable amount of an appropriate calcium salt.

17. A method of claim 11, wherein the suspension and the composition containing thrombin are applied to the site in a surgically acceptable vehicle.

18. A method of claim 17, wherein the vehicle comprises a gel.

19. A method of claim 17, wherein the vehicle comprises a stretched fabric.

20. A fibrin glue prepared by the method of claim 11.

21. A fibrin glue in a gel vehicle prepared by the method of claim 17.

22. A method of sealing a surgical wound which comprises applying to the wound a suitable amount of a cryoprecipitated suspension of claim 9 and applying a composition containing a sufficient amount of thrombin to the site so as to cause the fibrinogen in the suspension to be converted to a fibrin glue which then solidifies in the form of a gel.

23. A method of claim 22, wherein the suspension and composition are contained in a surgically acceptable vehicle.

24. A method of claim 23, wherein the vehicle comprises a gel or a stretched fabric.

25. A method of claim 24, wherein the stretched fabric comprises a synthetic vascular patch or graft.

26. A method of claim 22 which further comprises applying pressure to the fibrin glue for an effective time for the glue to solidify.

27. A fibrin glue kit for use in providing hemostasis during surgery which comprises a cryoprecipitated suspension of claim 9 and composition containing thrombin.

28. A kit of claim 27, wherein the composition containing thrombin contains a suitable amount of an anti-fibrinolytic substance.

29. A kit of claim 28, wherein the anti-fibrinolytic substance comprises apoprotinin.

30. A kit of claim 27, wherein the composition containing thrombin also contains a suitable amount of an appropriate calcium salt.

31. A kit of claim 27 which further comprises a surgically acceptable vehicle for the fibrin glue.

32. A kit of claim 31, wherein the vehicle is a gel.

33. A kit of claim 27 which further comprises at least one synthetic vascular patch.

34. A kit of claim 27 which further comprises at least one synthetic vascular graft.

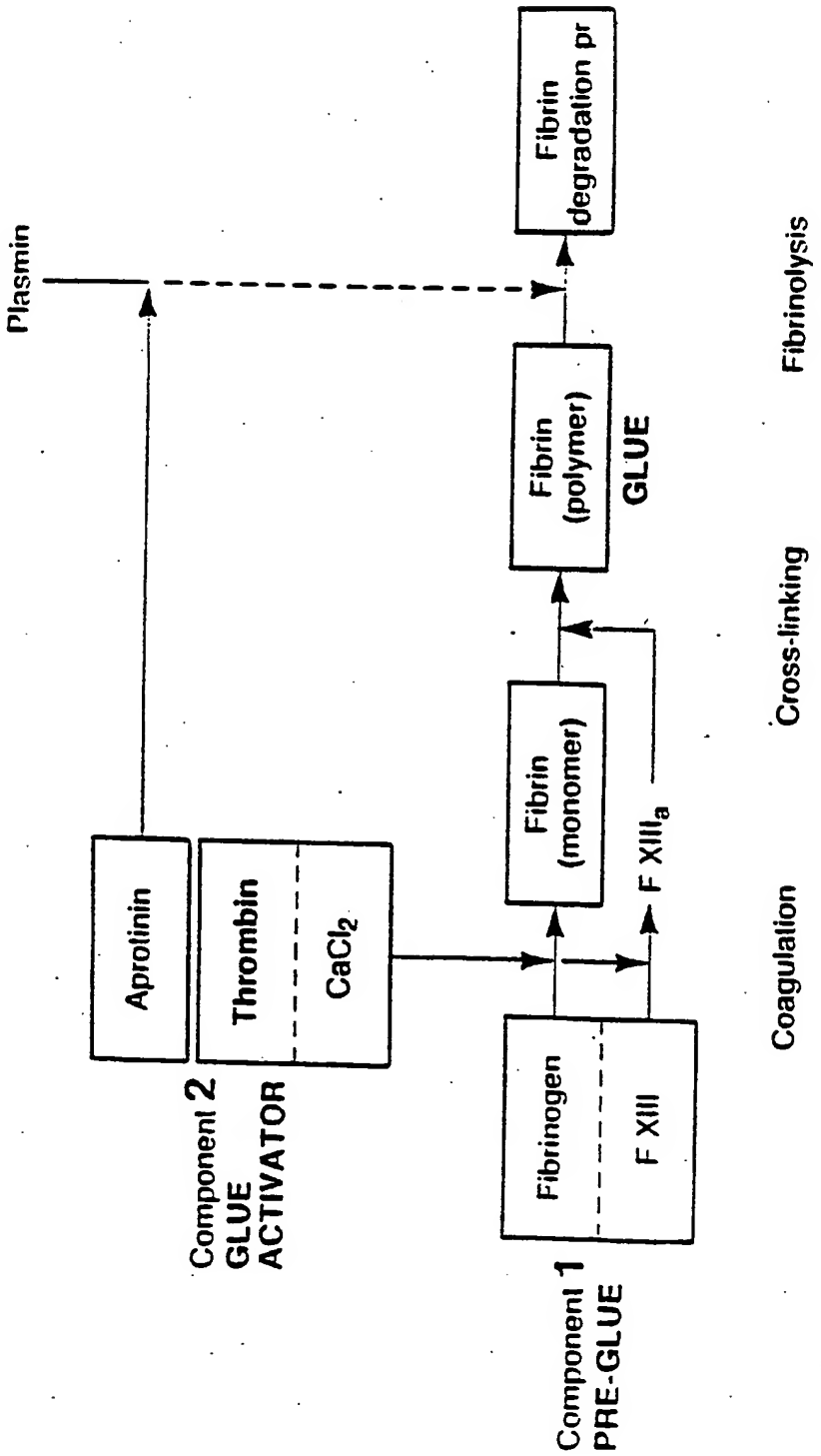
Fibrin degradation pr
Plasmin
Fibrinolysis
Fibrin (polymer)
GLUE
Cross-linking
Fibrin (monomer)
F XIII$_a$
Aprolinin
Thrombin
$CaCl_2$
Component 2
GLUE
ACTIVATOR
Coagulation
Fibrinogen
F XIII
Component 1
PRE-GLUE

FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/01695

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL. 4 C08L 89/00; A61K 35/14
U.S. CL. 106/124, 161; 128/334; 424/101

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U.S.	106/124, 161; 35; 436/511; 128/334; 424/ 101

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 5

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X	US, A, 2,112,496 PUBLISHED 29 MARCH 1938 IDE ET AL	1-10 & 27-34
X	US, A, 4,362,567 PUBLISHED 07 DECEMBER 1982 SCHWARZ ET AL	1-10 & 27-34
X	US, A, 4,414,976 PUBLISHED 15 NOVEMBER 1983 SCHWARZ ET AL	1-10 & 27-34
X	US, A, 4,419,938 PUBLISHED 22 MAY 1984 POLLAK	27-34
X	US, A, 4,427,650 PUBLISHED 24 JANUARY 1984 STROETMANN	1-27

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
12 NOVEMBER 1985	03 DEC 1985
International Searching Authority 1	**Signature of Authorized Officer 20**
ISA/US	T. MORRIS

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

SEE ATTACHMENT I

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

PCT/US85/01695

ATTACHMENT I

I. Claims 1-10 and 27-34, drawn to a cryoprecipitated suspension, classified in Class 106, subclass 124.

II. Claims 11-20, drawn to a method preparing a fibrin glue, classified in Class 424, subclass 28.

III. Claims 21-26, drawn to a method of sealing a surgical wound, classified in Class 128, subclass 334.